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SHORT COMMUNICATION

Impact of new ingredients obtained from brewer's spent yeast on bread characteristics

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Abstract The impact of bread fortification with β-glucans and with proteins/proteolytic enzymes from brewers' spent yeast on physical characteristics was evaluated. β-Glucans extraction from spent yeast cell wall was optimized and the extract was incorporated on bread to obtain 2.02 g β-glucans/100 g flour, in order to comply with the European Food Safety Authority guidelines. Protein/proteolytic enzymes extract from spent yeast was added to bread at 60 U proteolytic activity/100 g flour. Both β-glucans rich and proteins/proteolytic enzymes extracts favoured browning of bread crust. However, breads with proteins/proteolytic enzymes addition presented lower specific volume, whereas the incorporation of β-glucans in bread lead to uniform pores that was also noticeble in terms of higher specific volume. Overall, the improvement of nutritional/health promoting properties is highlighted with β-glucan rich extract, not only due to bread β-glucan content but also for total dietary fibre content (39% increase). The improvement was less noticeable for proteins/proteolytic enzymes extract. Only a 6% increase in bread protein content was noted with the addition of this extract and higher protein content would most likely accentuate the negative impact on bread specific volume that in turn could impair consumer acceptance. Therefore, only β-glucan rich extract is a promising bread ingredient.

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Introduction

Bread is consumed in large amounts on a daily base and has an important role in human nutrition. The increasing awareness of a healthy lifestyle is associated with consumers demand for breads with lower calories and more fibre. Consequently, breads containing functional ingredients became more important in the bakery industry and on the market. However, finding new functional ingredients for bread making that can improve both technological and nutritional/health properties is a challenging task.

Brewing spent yeast, the second major by-product from brewing industry can be a rich source of functional ingredients, such as fibre (mainly β-glucans), protein (including proteolytic enzymes), vitamins, and minerals (Ferreira et al. 2010; Petravić-Tominac et al. 2011).

Yeast β-glucans are water-insoluble and nondigestible cell wall polysaccharides that are able to modulate mucosal immunity of the intestinal tract, facilitate bowel motility, and can be used in obstipation, among other intestinal problems (Volman et al. 2008). The European Food Safety Authority (EFSA) has already approved the use of *Saccharomyces* β-glucans—referred to as "yeast beta-glucans"—as a new ingredient and suggests a use ranging between 50 and 200 mg of "yeast beta-glucans" per serving (EFSA 2011). Nerveless, the impact of "yeast beta-glucans" addition on bread characteristics is not known.

Yeast proteins and proteolytic enzymes are considered as Generally Recognized As Safe (GRAS) and present an adequate amino acid profile rich in essential amino acids, with sulphur-containing amino acids at levels above the

FAO/WHO reference (FAO/WHO 1990; Vieira et al. 2016). Therefore, the incorporation of yeast proteins in bread could potentially improve its nutritional quality. The use of exogenous enzymes in the baking industry is described to improve dough and bread quality and shelf life (Poutanen 1997). However, addition of proteolytic enzymes to bread can also have a negative impact on baking quality. No studies were found concerning the effect of spent yeast protein/enzymes incorporation on bread quality.

The objective of this work was to evaluate the impact of bread fortification with β-glucan rich extract and proteins/proteolytic enzymes from brewers' spent yeast on bread physical properties and quality.

Materials and methods

β-Glucan and proteins/proteolytic enzymes extraction from spent yeast

The spent yeast biomass obtained from Lager beer production (*Saccharomyces pastorianus*) was supplied by a local beer industry. β-Glucan rich extract was obtained from spent yeast (5 L, with solids content adjusted to 15% w/w and pH 5) through autolysis (50 °C for 24 h), alkaline extraction (five volumes of 1.0 N sodium hydroxide at 80 ± 5 °C for 2 h, and acid extraction (five volumes of 0.5 N acetic acid at 75 ± 5 °C for 1 h), as described by Thammakiti et al. (2004). The obtained β-glucan rich extract was lyophilized and later milled. β-Glucan content (19.79 ± 1.66 mg/100 g, dry weight) was quantified using the "Enzymatic yeast beta-glucan—assay procedure" (Megazyme International Ireland Ltd., Bray, Ireland). Additionally, β-glucan rich extract was characterised for its content in total dietary fibre (72.31 ± 2.03 g/100 g, dry weight) using commercially available kits (K-TDFR, Megazyme, Cork, Ireland) based on the methods of Prosky et al. (1985) and Prosky et al. (1988). Analyses on β-glucan rich extract were performed in triplicate.

For the extraction of protein/proteolytic enzymes from spent yeast, the mechanic disruption process described by Vieira et al. (2013) was adopted. The resulting proteins/proteolytic enzymes extract was lyophilized. Proteolytic activity (127.5 ± 40.0 mg leucine/h/g, dry weight), where one unit of activity (U) corresponds to the enzyme activity that liberates 1 mg leucine/h/g under the assay conditions, was determined according to Mäkinen and Arendt (2012). Total protein determination (64.1 ± 0.2 g/100 g, dry weight) was carried out by the Kjeldahl method (AOAC 2000), using factor conversion of 6.25. Analyses on proteins/proteolytic enzymes extract were performed in triplicate.

Bread samples

Bread samples were produced at a pilot scale in an experimental laboratory (Ceres, Porto, Portugal). Wheat flour type 650 supplied by Ceres (Porto, Portugal) had the following composition: 14.5 g of moisture, 11 g of protein, 73 g of carbohydrates, 3.5 g of fibre, and 1.6 g of fat per 100 g of flour with a falling number of 220 s. Fresh yeast and salt used in this study were also supplied by Ceres (Porto, Portugal). Control bread (BC) and breads fortified with β-Glucans (BβG) and proteins/proteolytic enzymes (BPT) were prepared following the recipes shown in Table 1. EFSA maximum recommendations on yeast β-glucan (200 mg/dose) were considered for the incorporation of β-glucan rich extract. In addition, this incorporation represented a 39% increase in bread total dietary fibre, comparing to the BC. For incorporation of proteins/proteolytic enzymes extract, maximum values used by Mäkinen and Arendt (2012) were considered i.e., 60 U. Furthermore, this incorporation represented a 6% increase in bread protein content, comparing to the BC. Bread ingredients were mixed and kneaded for 20 min. Each 65 g of dough was then shaped into a ball, proofed for 90 min at 30 °C with 80% relative humidity, and baked for 10 min at 200 °C. Bread samples were cooled 90 min at room temperature before further analysis. Six replicates were analysed for each bread formulation.

Evaluation of breads physical characteristics

Weight, specific volume, and moisture

Breads weight (P) and specific volume (SV) were measured cooling (n = 6). Bread SV was measured using a seed displacement method (Cerealis internal method) and the following equation

$$SV = \frac{1.35S}{P} \quad (1)$$

Table 1 Recipe for each bread formulation

Ingredients (g)	Bread formulations		
	BC	BβG	BPT
Wheat flour	1500	1500	1500
Salt	30	30	30
Yeast	75	75	75
Water	900	900	900
β-Glucan rich extract	–	30.7	–
Proteolytic enzymes extract	–	–	4.7

BC control bread, *BβG* bread fortified with β-Glucans and *BPT* bread fortified with proteins/proteolytic enzymes (BPT)

S (g), Weight of the displaced seeds; 1.35 (cm^3/g), Specific volume of the *Phalaris canariensis* seeds.

Crumb structure image analysis

To study the crumb structure, six slices from every bread formulation were cut (1.6 cm thickness). Each slice was positioned on the flatbed scanner (Canon iR2016i, Netherlands) in pre-standardized conditions (black cardboard box over the slice, in order enhance contrast) (Russ 2011). Images were captured in the RGB (24 bit) standard format with a resolution of 300 dpi and saved in JPG format. Each image was processed and analysed using Matlab R2015a (MathWorks). A single 200 × 200 pixel (85x85 mm) field of view was cropped and converted to a 256 level grey scale. Image segmentation was performed with Otsu's method (Otsu 1979) and cell morphological parameters were analysed with Matlab R2015a (MathWorks). Resulting data included: number of cells, mean cell area (mm^2), and cell density ($cells/mm^2$). Additionally, cells were separated into different classes as a function of their area: very small size ($0.2\ mm^2 \leq$ cell area); small size ($0.2 <$ cell area $\leq 3.0\ mm^2$); medium size ($3.0 <$ cell area $\leq 10.0\ mm^2$); large size (cell area $> 10.0\ mm^2$).

Evaluation of bread colour

For colour analysis, six breads of every formulation were cut in half and the measurements were performed in three different points on the crumb and crust. Colour measurement was done in the CIElab system using a Minolta CR-300 colorimeter (Minolta, Ramsey, NJ) with illuminant D65, a 0° standard observer and a 2.5 cm port/viewing area. Before colour measurements in crust and crumb, the colorimeter was calibrated with a white tile having the following values: L* = 93.5, a* = 1.0 and b* = 0.8 before L*, a* and b*. Additionally, crumb L*, a* and b* values were combined in the browning index (BI) parameter (Buera et al. 1985) according to Eqs. 2 and 3

$$BI = \frac{100(X - 0.31)}{0.172} \quad (2)$$

$$X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \quad (3)$$

Statistical analysis

Incorporation of β-glucan rich extract and proteins/proteolytic enzymes extract were studied using a one-way ANOVA (post-hoc tests: Tukey´s or Tamhane's T2) or Kruskal–Wallis test (post-hoc test: Dunn's), according to the residuals distribution. All statistical analyses were conducted with the XLSTAT for Windows version 2016.02 (Addinsoft, Paris, France) at 5% significance level.

Results and discussion

Bread weight, specific volume, and moisture

As seen in Table 2, bread weight and specific volume varied significantly between different formulations. While BβG registered the lowest ($p < 0.005$) weight, BPT presented the lowest ($p < 0.05$) specific volume. A reduction in specific volume could occur by interference or impairment of the gluten network development due to the presence of gluten degrading enzymes (proteases) (Caballero et al. 2007). Also, the protein source and functionality play an important role (Zhou et al. 2017). Regarding bread moisture, no significant differences were registered between different formulations.

Bread colour and morphological features

The effect of bread fortification with β-Glucans or with proteins/proteolytic enzymes on appearance and crumb structure illustrated in Fig. 1. β-Glucans and proteins/proteolytic enzymes fortification had a different impact on appearance and crumb structure when compared with BC. BβG presented high height and a golden color than BC and BPT.

Instrumental analysis indicated that crust and crumb colour of different bread formulations varied considerably (Table 2). Regarding crumb L*, BPT presented the lowest values (darker) while BβG the highest. A decrease in crumb L* was also observed by Martins et al. (2015) with the addition of extract recovered from brewing spent yeast. Every bread formulation presented a reddish (positive a*) bread crumb. BβG crumb a* values were significantly lower from BC and BPT. Crumb b* values were positive for every bread formulations (yellowish colour). The highest b* values were observed with BβG and the lowest with BPT. In relation to bread crust colour parameters, crust L* was significantly lower (darker) for BPT than for other formulations. Besides, no significant differences were detected between BC and BβG. Bread crust exhibited a reddish colour (positive a*) for every bread formulation. BβG and BPT crust a* values were significantly higher than BC. Crust b* was positive for every bread formulations that exhibited a yellowish colour. The highest b* values were found for BβG and BPT, while the lowest were for BC. Browning in bread crust was favoured in BβG and BPT, which presented significantly higher BI values than BC. The BI is an important bread crust parameter to follow the evolution of Maillard and caramelisation reactions,

Table 2 Values for physical parameters, colour, and crumb image analysis for bread with different formulations

Physical parameters	Bread sample			*P*
	BC	BβG	BPT	
Weight (g)	47.1 ± 1.5[a]	45.0 ± 1.0[b]	47.0 ± 1.8[a]	0.041*
Specific volume (cm^3/g)	3.0 ± 0.2[a]	3.2 ± 0.2[a]	2.3 ± 0.1[b]	< 0.001**
Moisture (%)	28.87 ± 1.25	24.62 ± 3.27	26.51 ± 1.94	ns
Crumb				
L* (–)	61.91 ± 3.34[a]	64.85 ± 2.84[b]	57.72 ± 3.23[c]	< 0.001*
a* (–)	0.57 ± 0.22[a]	0.42 ± 0.21[b]	0.64 ± 0.23[a]	< 0.001*
b* (–)	15.45 ± 0.72[a]	17.98 ± 0.64[b]	15.95 ± 0.64[c]	< 0.001*
Crust				
L* (–)	71.62[a] (60.83–75.32)	71.01[a] (62.89–76.31)	66.37[b] (58.22–71.19)	< 0.001***
a* (–)	3.23 ± 1.17[a]	4.54 ± 1.47[b]	5.27 ± 1.55[b]	< 0.001*
b* (–)	25.07 ± 3.00[a]	27.25 ± 2.73[b]	26.72 ± 2.23[b]	0.002*
BI (–)	46.73 ± 8.24[a]	52.01 ± 11.83[b]	57.31 ± 8.81[b]	< 0.001*
Number of cells (–)	380 ± 50	384 ± 80	373 ± 43	ns
Mean area (mm^2)	9.87 ± 0.88	10.29 ± 2.51	9.69 ± 0.55	ns
Cell density ($cells/mm^2$)	38.69 ± 6.01	40.17 ± 14.88	37.41 ± 8.05	ns
Cell area (% of total cells)				
$0.2 \leq CA$ (mm^2)	41.02 ± 2.34[ab]	43.99 ± 0.37[a]	39.85 ± 2.07[b]	0.018*
$0.2 < CA \leq 3.0$ (mm^2)	44.41 ± 1.89	43.62 ± 2.29	45.06 ± 2.31	ns
$3.0 < CA \leq 10.0$ (mm^2)	7.01 ± 1.81	6.22 ± 1.57	7.56 ± 0.99	ns
$CA > 10.0$ (mm^2)	8.00 ± 0.32	6.42 ± 2.65	7.97 ± 0.35	ns

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution (n = 6)

BC control bread, *BβG* bread fortified with β-Glucans, *BI* browning index, *BPT* bread fortified with proteins/proteolytic enzymes and *CA* cell area

p Values from one-way ANOVA analysis. Means were compared by Tukey's test, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$)

**p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$)

***p* Values from Kruskal–Wallis analysis. Medians were compared by Dunn's test

which are responsible for the brown colour formation (Ramírez-Jiménez et al. 2000). However, comparison with literature is not possible, since bread colour depends on factors such as formulation or baking conditions.

Crumb morphological features of the different bread formulations are compared in Table 2. Crumb morphology is an important bread quality parameter alongside taste, crumb colour and crumb texture (Skendi et al. 2010; Zayas 1993). Regarding number of cells, mean area, and cell density, only minor variations were observed. When observing cell area distribution, more than 84% of cells in every bread formulation had an area below 3.0 mm^2. Nevertheless, significant differences in cell area distribution were only found for very small size class (cell area $\leq$ 0.2 mm^2), between BβG and BPT. BβG had higher percentage of cells pertaining to this class than BPT; this was also reflected in terms of higher specific volume and is in agreement with Gallagher et al. (2003). β-Glucans extracted from yeast could have an impact similar to oats, barley or rye β-glucans and could be involved in gas cells stabilization in the dough and prevent their coalescence (Lazaridou and Biliaderis 2007; Polaki et al. 2010; Wang et al. 1998). This resulted in the development of smaller and more uniform crumb bread pores, which in turn affected the bread quality positively (Polaki et al. 2010; Wang et al. 1998).

Conclusion

Overall, β-glucan rich and proteins/proteolytic enzymes extracts did not compromise most of the bread physical parameters evaluated in this study, when compared to the control. However, β-glucan rich extract stands out

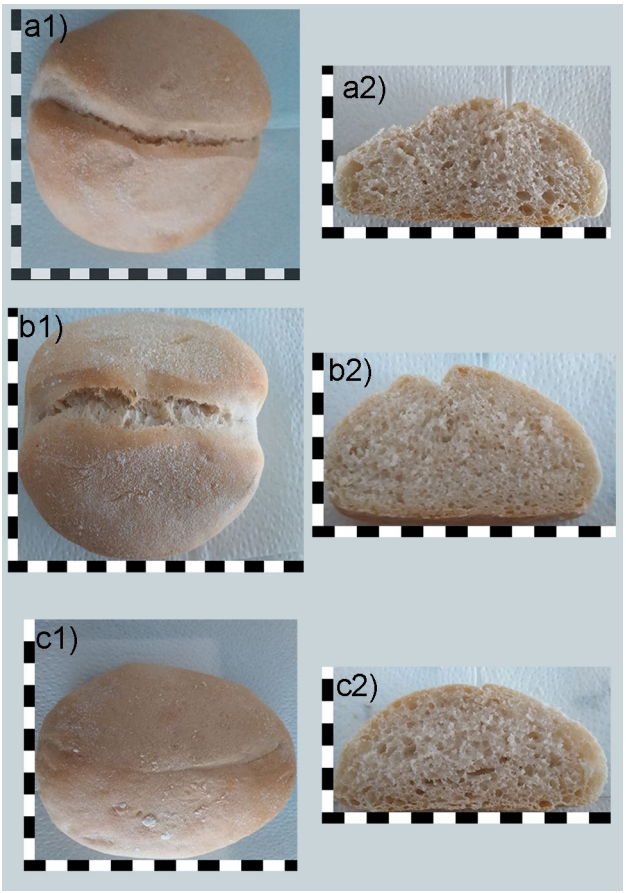

Fig. 1 Overall observations (1) and cross-sections (2) of breads. **a** Control bread (BC), **b** bread fortified with β-glucans (BβG), **c** bread fortified with proteins/proteolytic enzymes (BPT). One rectangle in the scale is 1 cm (width, height)

regarding improvement of nutritional/health promoting properties, not only for bread β-glucan content but also for total dietary fibre content (39% increase). As for proteins/proteolytic enzymes extract, nutritional/health promoting properties was less notorious, with only a 6% increase in bread protein content. Although proteins/proteolytic enzymes extract incorporation only had a negative impact on bread specific volume, an increase in incorporation level would most likely accentuate this effect. In this case, nutritional/health promoting properties would not compensate a detrimental impact on physical properties that in turn could impair consumer acceptance. Therefore, only β-glucan rich extract seems to be a promising functional ingredient to improve bread nutritional/health promoting properties without compromising quality.

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